METHODS SUPPLEMENT

Yeast strains and plasmids: The strains used for this study were derived from W303[1] and are listed in Table 1. Strain LSY1092 was made by one-step gene replacement of W1588-4A with *Eco*RI/*Sph*I-digested pMJ536 (gift of M. Lichten) to replace the *SAE2* coding region with the kanMX6 cassette[2]. G418 resistant transformants were tested for sensitivity to methyl methane sulfonate and confirmed by Southern blot analysis. Strain LSY1975 was constructed by one-step replacement of W1588-4C with a PCR fragment to generate a deletion allele of *SGS1.* PCR was performed on an *hph*MX4 cassette plasmid (pAG32, kindly provided by J. McCusker)[3], with the following primers: 5-ATTATTGTTGTATATATTTAAAAAATCATACACGTACACACAAGGCGGTAATGCGTACGCTGCAGGTCGAC and 5-TTGGCGAATGGTGTCGTAGTTATAAGTAACACTATTTATTTTTCTACTCTTCAATCGATGAATTCGAGCTCG. Underlined letters indicate the bases in plasmid sequences, and the rest indicate the bases in *SGS1* sequences. Strain YAR1006 was kindly provided by A. Rattray. To create strain LSY2041, a PCR fragment amplifying the tet-*SAE2* cassette was transformed into W1588-4C. PCR was performed on YAR1006 genomic DNA using the following primers: 5'- CAAAATCAAGTCAAATAGCC and 5'- CATTTATTCCGATTTTGCAG. A PCR fragment amplifying a 13-Myc tag was transformed into LSY2041 to generate strain LSY2077. PCR was performed on a 13Myc containing plasmid, pFA6a-13Myc-*TRP1*[4] with the following primers: 5'-GACGATGGATGTTTCTTCTGGAGTGATAAATTATTGCAGATATATGCTAGATGTCGGATCCCCGGGTTAATTAA and 5'-ATACCAAAAAAAATGTATTTGAAGTAATGAATAAAGAATGATGATCGCTGGCGTGAATTCGAGCTCGTTTAAAC. Underlined letters indicate the bases in plasmid sequences, and the rest indicate the bases in *SAE2* sequences. Strains containing the *ade2* direct repeat were

generated by crossing LSY1430[5] to strains within the lab collection to produce haploid progeny of the indicated genotypes.

Plasmids containing the wild type or helicase defective *SGS1*, pSM100 and pRS415-*sgs1-K706A,* respectively, were kindly provided by R. Rothstein. pEM-*SGS1* and pEM-*sgs1-K706A* were created by moving the *SGS1* containing *KpnI/SacI* fragment from plasmids pSM100 and pRS415-*sgs1-K706A* respectively, into the multiple cloning site of pRS316.

Media, growth conditions, and genetic methods. Media, growth conditions and genetic methods are as described previously[6]. Hygromycin B (Sigma) to 300 μg/ml was used for selection of the *hph*MX4 cassette. Doxycycline (Sigma) was used to 20 μg/ml final concentration in the conditional Sae2 experiments. G418 (Sigma) to 200 μg/ml was used for selection of the *kan*MX cassettes. Yeast strains were grown at 30° unless otherwise indicated.

Physical analysis of mating type switching. Strains to be tested were transformed to Trp^+ with HO expressing plasmid, pFH800[7]. Trp^+ plasmid-containing transformants were grown in 5 ml of SC medium lacking tryptophan (SC-Trp) for 18 h. Cells were harvested, washed with water and used to inoculate 200 ml of SCRaf-Trp. Cultures were grown to an OD_{600} of 0.3 to 0.4, a 50ml sample was removed (t=0h) and 16.8ml galactose was added to the medium to a final concentration of 2%. One hour later, the cultures were harvested, washed and resuspended in 200 ml of SCRaff-Trp + 2% glucose. Samples (40 ml) were removed at 1-h intervals after induction for DNA analysis. Cells were harvested by centrifugation and washed with water, and the cell pellets were stored at -20°. DNA was extracted, digested with *Sty*I, and DNA fragments were separated by electrophoresis through 1% agarose gels. DNA fragments were transferred to nylon membranes and hybridized with a 404-bp PCR fragment

generated by amplification of *MAT* sequences distal to the HO-cut site (coordinates 201176 to 201580 on the ChrIII sequence). For the conditional Sae2 experiments, the same protocol was followed with the exception that before addition of galactose, the cells were pre-treated with 20 μg/ml doxycycline for 6h to ensure full repression of Sae2 expression.

To quantitate the repair efficiency we compared the intensity of the gene conversion product to the total intensity of the lane, using ImageJ. This ratio was then corrected for the HO cutting efficiency for each experiment. To quantitate the cutting efficiency we made use of a set of primers that specifically anneal to Ya and Z2 sequences and amplify only the uncut substrate. We specified the PCR conditions required for log-linear region amplification and performed PCR using as templates genomic DNA from t=0h and t=2h. In the same PCR reaction a second set of primers with the same annealing temperature but unrelated target locus were used as loading control. The PCR products were analyzed on agarose gels and the intensity of the bands was quantitated using ImageJ. For each time point we calculated the ratio of uncut MAT/control, designated as x. The ratio of x_{2h}/x_{0h} provides us the percentage of uncut MATa that when subtracted from 1, gives the cutting efficiency.

Physical analysis of single strand annealing: Strains to be tested were grown in 5 ml of SC-Trp for 18 h. Cells were harvested, washed with water and used to inoculate 200 ml of YPLactate (3%) medium. Cultures were grown to an OD_{600} of 0.3 to 0.4, a 50ml sample was removed (t=0h) and 16.8ml of 20% galactose were added to the medium. One hour later, the cultures were harvested, washed and resuspended in 200 ml of YPLactate + 2% glucose. Samples (40 ml) were removed at 1-h intervals after induction for DNA analysis. Cells were harvested by centrifugation and washed with water, and the cell pellets were stored at -20°. DNA was extracted, digested with *Nhe*I/*Eag*I, and DNA fragments were separated by electrophoresis through 0.8% agarose gels. DNA fragments were transferred to nylon membranes and hybridized with a 3.7kb *Bgl*II fragment of the *ADE2* locus (coordinates

563761 to 567474 on the chromosome XV sequence). The final SSA product was quantitated by the ratio of the intensity of the SSA product to the whole intensity of the lane, using ImageJ. For the conditional Sae2 experiments, the same protocol was followed with the exception that before addition of galactose, the cells were pre-treated with 20 μg/ml doxycycline for 6h.

Dot blot analysis: DNA samples from the SSA induction experiment were applied to nylon membranes in the native or denatured state. 1 μg versus 0.1 μg of total genomic DNA was used for the native and denatured samples, respectively, after adjusting the concentration to 10xSSC. Denaturation was achieved by boiling the samples for 5min and rapidly cooling on ice for 5min. Samples were loaded into the wells of a dot blot apparatus, transferred to nylon membranes and the wells were rinsed with 10xSSC. The membrane was UV cross-linked and the blots were hybridized with the *ADE2* probe. The signal of the dots was quantified by scanning the optic density of each dot using ImageJ software (NIH, USA).

Alkaline electrophoresis: For analysis of ssDNA intermediates, DNA fragments were separated on alkaline gels as described[8] and the blots hybridized with single stranded probes complementary to the 5' or 3' strand. The probes were obtained by in vitro transcription using Epicentre Riboscribe T7 synthesis system and plasmids pEM-MAT or pEM-TAM as templates. The plasmids were obtained by cloning a *MAT* locus PCR fragment (coordinates 201176 to 201580 on the ChrIII sequence) into pGEM-T Easy (Promega) in either orientation. Forward orientation gave rise to pEM-MAT and was used as a template for synthesis of a ribo-probe to detect 3' strand intermediates. Reverse orientation gave rise to pEM-TAM that was used as a template for synthesis of a ribo-probe to detect 5' strand intermediates. To quantitate the r2 band over time in different strain backgrounds we plotted the ratio of intensity of the r2 band to the intensity of an unrelated locus as a loading control,

using ImageJ.

Western blot analysis: Whole cells extracts were analyzed by SDS-PAGE electrophoresis and Sae-Myc was detected by using anti-c-myc antibody (Sigma).

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TABLE 1. Yeast strains

Strain	Genotype[a]	Source
W1588-4C	*MAT***a**	R. Rothstein
W1588-4A	*MAT*α	R. Rothstein
YHK595-3B	*MATa rad51::LEU2*	H. Klein
LSY0615[b]	*MAT*α *mre11::LEU2*	[9]
LSY0624	*MAT***a** *exo1::URA3*	[10]
LSY1092	*MAT*α *sae2::KANMX6*	This study
LSY1430	*MAT***a** *ade2-n::TRP1::ade2-I exo1::HIS3*	[5]
LSY1709-9D	*MAT***a** *rad51::LEU2 ade2-n::TRP1::ade2-I lys2::GAL-ISCEI*	This study
LSY1709-4A	*MAT*α *rad51::LEU2 exo1::HIS3 ade2-n::TRP1::ade2-I lys2::GAL-ISCEI*	This study
LSY1709-12D	*MAT***a** *exo1::HIS3 ade2-n::TRP1::ade2-I lys2::GAL-ISCEI*	This study
LSY1833-37B	*MAT*α *rad51::LEU2 exo1::HIS3 sae2::KANMX6 ade2-n::TRP1::ade2-I lys2::GAL-ISCEI*	This study
LSY1880-31A	*MAT*α *rad51::LEU2 sae2::KANMX6 ade2-n::TRP1::ade2-I lys2::GAL-ISCEI*	This study
LSY1924-48D	*MAT*α *rad51::LEU2 mre11::klURA3 ade2-n::TRP1::ade2-I lys2::GAL-ISCEI*	This study
LSY1924-7B	*MAT***a** *rad51::LEU2 mre11::klURA3 sae2::KANMX6 ade2-n::TRP1::ade2-I lys2::GAL-ISCEI*	This study
LSY1924-31D	*MAT*α *rad51::LEU2 mre11::klURA3 exo1::HIS3 ade2-n::TRP1::ade2-I lys2::GAL-ISCEI*	This study
LSY1924-35B	*MAT*α *rad51::LEU2 mre11::klURA3 exo1::HIS3 sae2::KANMX6 ade2-n::TRP1::ade2-I lys2::GAL-ISCEI*	This study
LSY1975	*MAT***a** *sgs1::HPHMX4*	This study
LSY1983-16B	*MAT***a** *rad51::LEU2 sgs1::HPHMX4 ade2-n::TRP1::ade2-I lys2::GAL-ISCEI*	This study
LSY1983-32B	*MAT*α *rad51::LEU2 sgs1::HPHMX4 exo1::HIS3 ade2-n::TRP1::ade2-I lys2::GAL-ISCEI*	This study
LSY1995-8C	*MAT*α *sgs1::HPHMX4 exo1::HIS3 ade2-n::TRP1::ade2-I lys2::GAL-ISCEI*	This study
LSY2010-10B	*MAT***a** *sgs1::HPHMX4 ade2-n::TRP1::ade2-I lys2::GAL-ISCEI*	This study
LSY2011-57C	*MAT***a** *ade2-n::TRP1::ade2-I lys2::GAL-ISCEI*	This study
LSY2012	*MAT***a** *sgs1::HPHMX4 exo1::HIS3*	This study
YAR1006[c]	*MAT***a** *KANMX-tTA-TetP::SAE2 ade2-101 his3-200 leu2-1 trp1-63 ura3-52*	A. Rattray
LSY2041	*MAT***a** *KANMX-tTA-TetP::SAE2*	This study
LSY2051-25D	*MAT***a** *rad51::LEU2 sgs1::HPHMX4*	This study
LSY2051-33D	*MAT***a** *rad51::LEU2 sgs1::HPHMX4 exo1::HIS3*	This study
LSY2051-26B	*MAT***a** *sgs1::HPHMX4 KANMX-tTA-TetP::SAE2*	This study
LSY2051-21D	*MAT***a** *sgs1::HPHMX4 exo1::HIS3 KANMX-tTA-TetP::SAE2*	This study
LSY2051-77B	*MAT*α *rad51::LEU2 sgs1::HPHMX4 exo1::HIS3 KANMX-tTA-TetP::SAE2 ade2-n::TRP1::ade2-I lys2::GAL-ISCEI*	This study

LSY2076-19A	*MAT***a** *rad51::LEU2 exo1::HIS3*	This study
LSY2077	*MAT***a** *KANMX-tTA-TetP::SAE2-MYC*	This study

[a]All strains listed except the ones noted are of the W303 genotype (*trp1-1 his3-11,15 can1-100 ura3-1 leu2-3,112 ade2-1 RAD5*). Only the mating type and differences from this genotype are shown

[b] *rad5-535*

[c] Not of the W303 background

SUPPLEMENTARY FIGURE LEGENDS

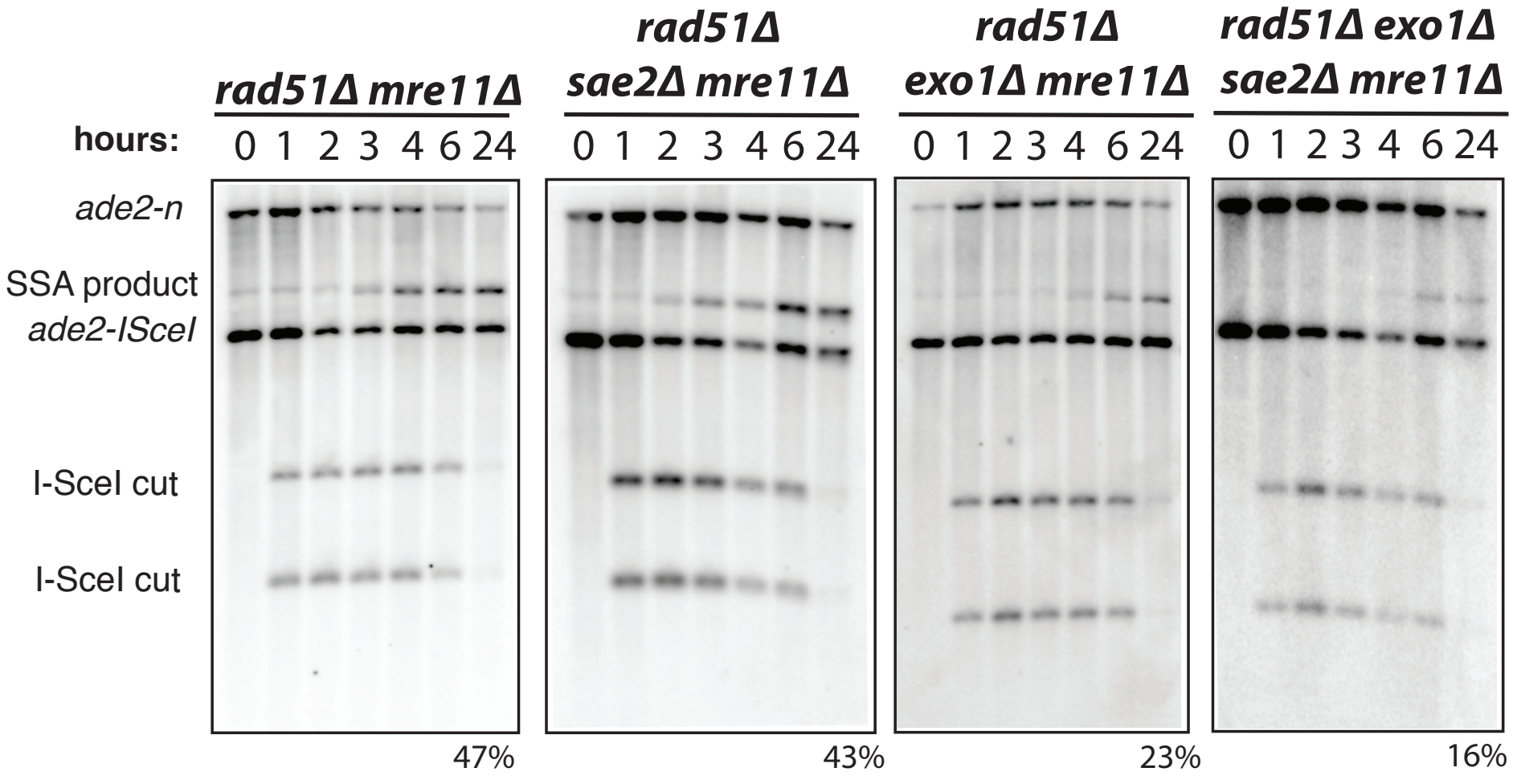
Supplementary Figure 1: ***mre11* is synergistic with *exo1* and epistatic to *sae2*.** SSA assay of the indicated strains. DNA samples from cells grown in YPLactate were prepared before and after a pulse (1h) induction with galactose, digested with *Nhe*I/*Eag*I, electrophoresed under native conditions, blotted onto nylon membrane and probed with the *ADE2* probe.

Supplementary Figure 2: **Quantitation of the SSA product efficiency after induction of I-*Sce*I in different strain backgrounds.** The relative SSA product intensity at each time point represents the intensity of the SSA band divided by the intensity of the loading control band from an unrelated locus, during a representative time course experiment.

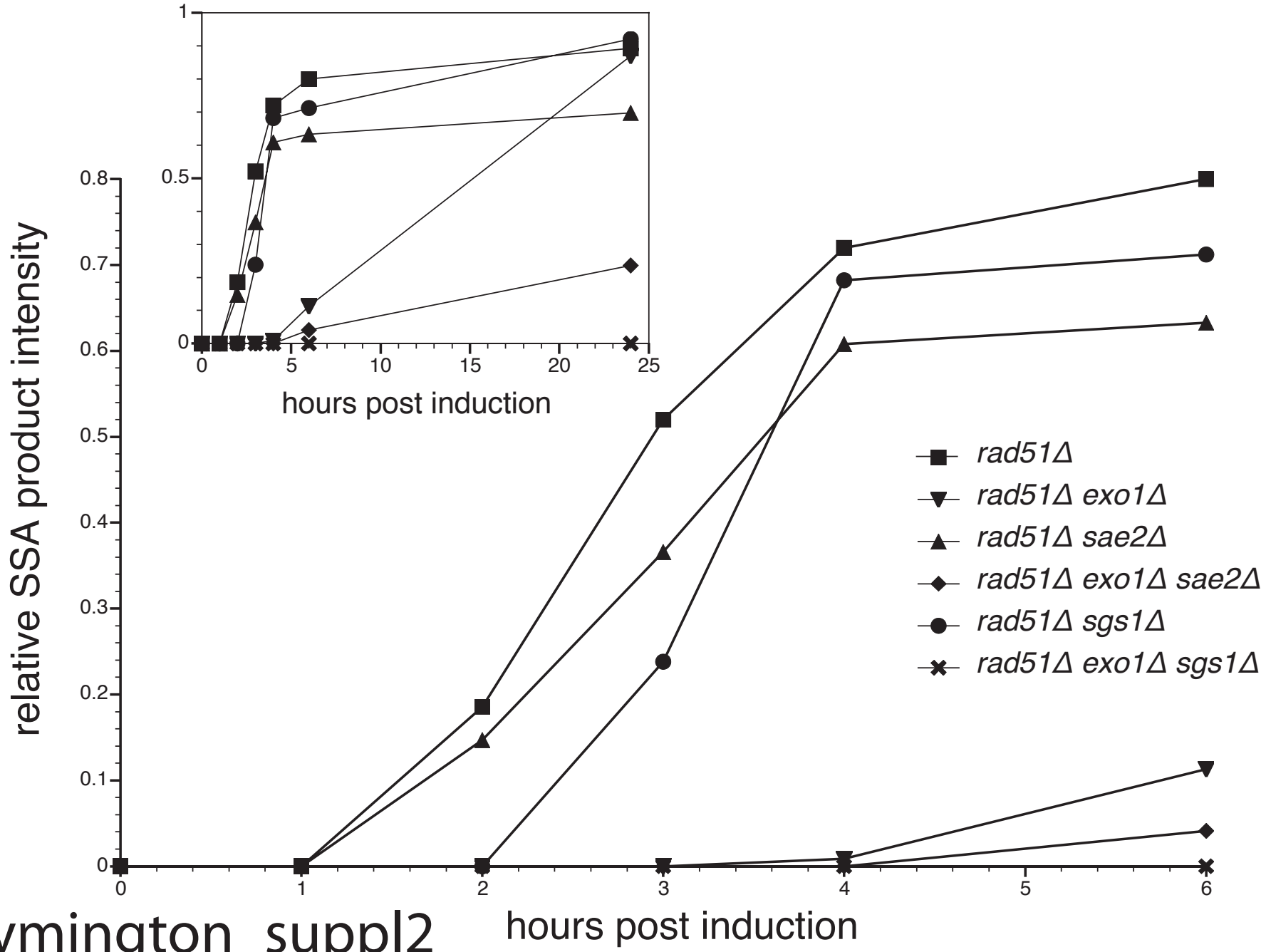
Supplementary Figure 3: **Gene conversion is efficient in the absence of Exo1 and/or Sae2.** a. Mating type switching assay: DNA samples prepared from cells before and after a pulse (1h) induction with galactose were digested with *Sty*I, electrophoresed under native conditions, blotted onto nylon membrane and probed with a *MAT* probe. b. Genomic DNA prepared from samples at the indicated time points before and after continuous induction with 2% galactose, was digested with *Sty*I and *Bst*XI, separated on alkaline-agarose gels and analysed by southern blot with a 3' strand specific ribo-probe.

Supplementary Figure 4**: Accumulation of the r2 ssDNA intermediate in the *rad51 exo1* mutant.** The plot represents the ratio of the r2 band intensity over loading control intensity, during a representative time course experiment. An accumulation of r2 is observed at later time points in the *rad51 exo1* mutant compared to *rad51* and *rad51 sgs1* strains.

Supplementary Figure 5: **Loss of Sae2 or Mre11 in *exo1 sgs1* background is lethal.** a. Spores derived from a diploid heterozygous for *EXO1, SGS1* and *SAE2* (LSY2012 x LSY1092). b. Spores derived from a diploid heterozygous for *EXO1, SGS1* and *MRE11* (LSY2012 x LSY0615). c. Western blot analysis of whole cell extracts after addition of doxycycline using anti-MYC antibody. d. Conditional loss of Sae2 in the absence of Sgs1 does not abolish gene conversion. Exponentially growing cells were left untreated or treated with 20 μg/ml doxycycline. Six hours later (t=0h), HO expression was induced by addition of galactose for an hour. At t=1h the cells were washed and resuspended in glucose containing medium. DNA samples prepared from cells before and after induction were digested with *Sty*I, electrophoresed under native conditions, blotted onto nylon membrane and probed with a *MAT* probe.



Symington_Suppl1



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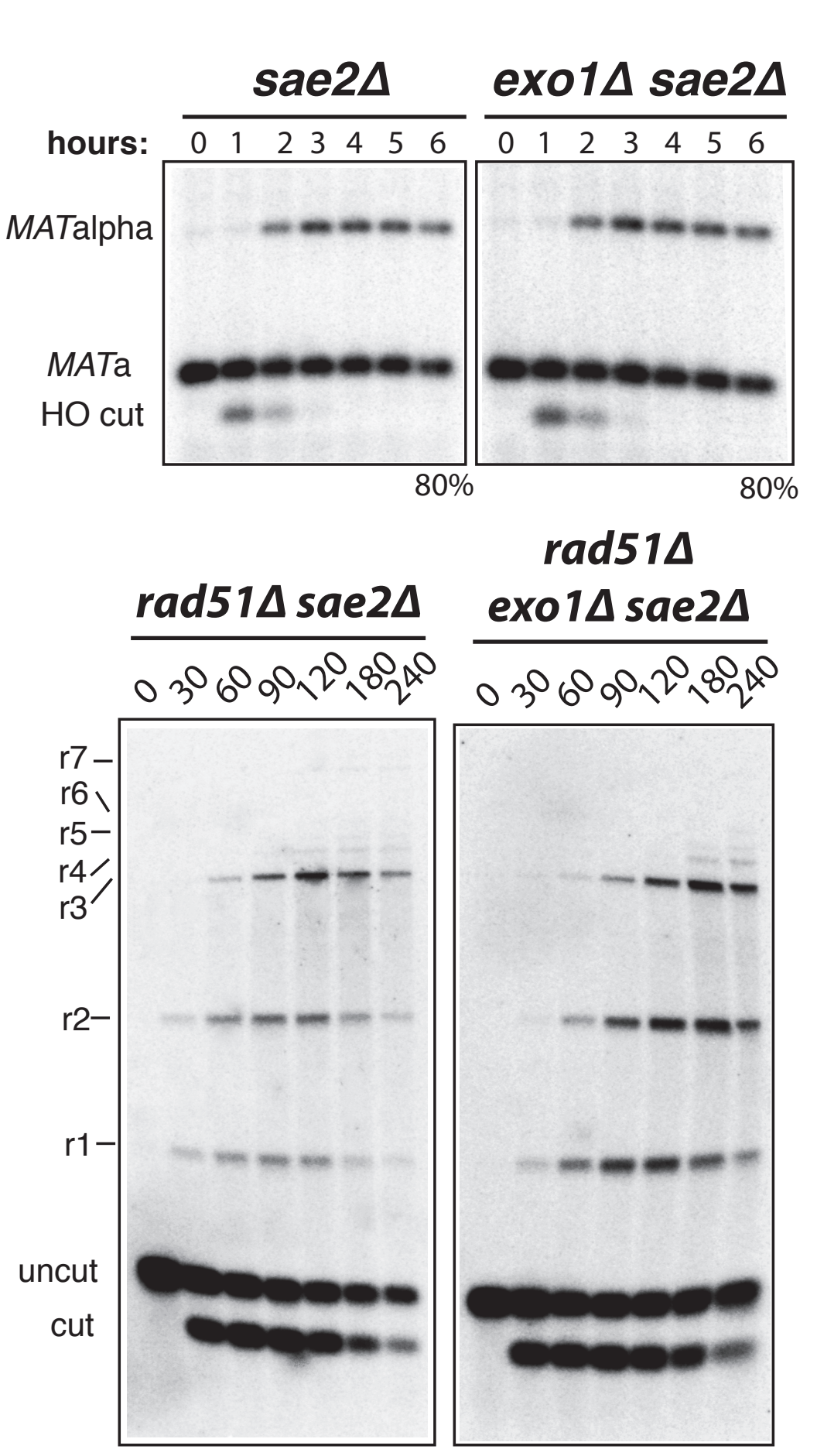

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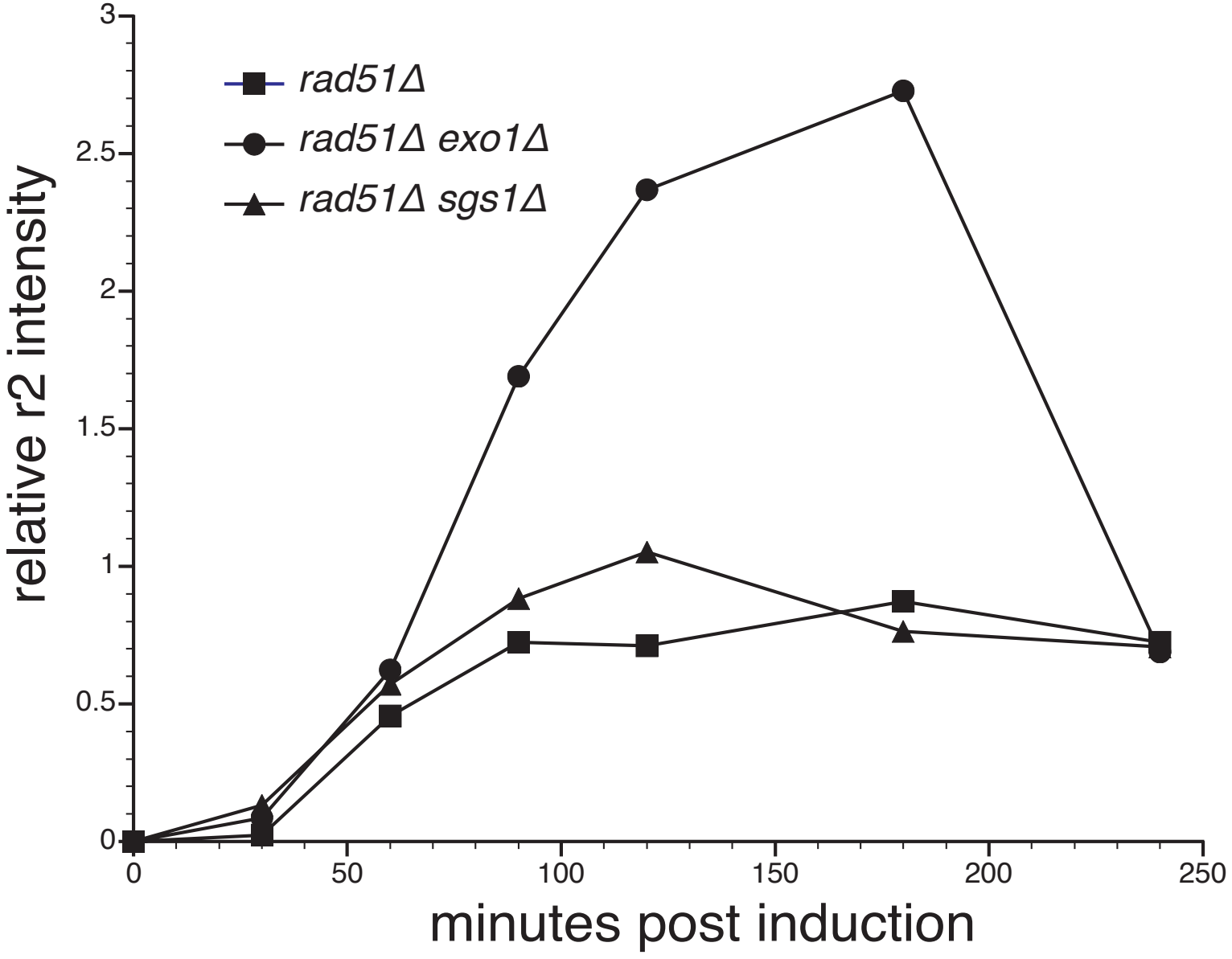

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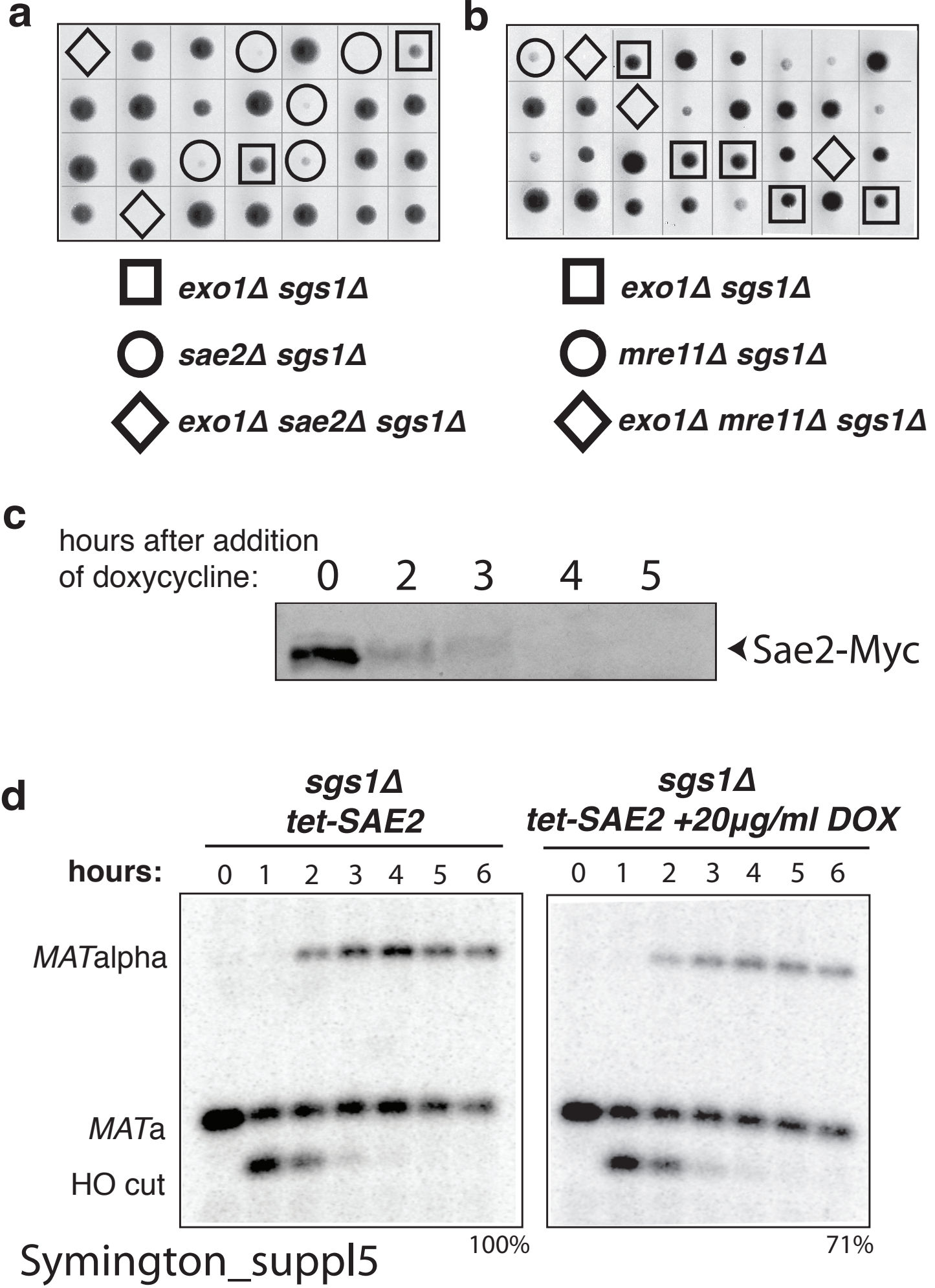

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